

Basophil Activation Test and specific IgE measurements using a panel of recombinant natural rubber latex allergens to determine the latex allergen sensitization profile in children

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There are no documented studies that describe natural rubber latex (NRL) sensitization in children with a history of surgical intervention but without any congenital malformation (urogenital anomalies, spina bifida, etc.), although some authors have studied NRL allergy in children without a history of surgical intervention. The aim of this work was to evaluate the sensitization profile to single NRL allergens in children without spina bifida and without repeated surgical interventions, by using different recombinant and natural latex allergens in two analytical techniques: specific serum immunoglobulin E (IgE) quantification and flow cytometry determination of activated basophils expressing CD63, after stimulating cells from patients with NRL allergens. A total of 23 patients and 10 healthy children were selected. Conjunctival and in-use NRL provocation tests were carried out, as well as specific IgE determination in all patients' and controls' sera with the recombinant NRL allergens: rHev b 1, rHev b 2, rHev b 3, rHev b 5, rHev b 6.01, rHev b 6.02, rHev b 8, rHev b 9 and rHev b 11 and with NRL (k82) using appropriate ImmunoCAPs. The Basophil Activation Test (BAT) was performed with whole latex extract and with the recombinant allergens rHev b 5 and rHev b 6.01, as well as with the natural allergen Hev b 6.02. The sensitivity and the specificity of NRL-specific IgE (k82) were 100%. Positive IgE responses to rHev b 5 were found in sera of 10 children, to rHev b 6.01 in 16 and for rHev b 6.02 in 15 children's sera. Specific IgE to rHev b 8 was found in four sera of the children. We only found significant differences in sensitization to rHev b 5 in children with two or more surgical interventions compared with the non-intervened group or those with only one intervention. Specific IgE in sera of children with latex-fruit syndrome recognized rHev b 6.02, but not to rHev b 11. The patients sensitized to Hev b 8, Hev b 9 and/or Hev b 11 were atopic. The four patients presenting a positive response to the NRL profilin Hev b 8 were allergic to pollen. The BAT against whole NRL extract was positive in 22 of 23 children; against rHev b 5 in 14 of the patients studied; against rHev b 6.01 in seven cases and against nHev b 6.02 in 19 children. In all the control subjects, the results using this technique were negative. If combined rHev b 5, rHev b 6.01 and nHev b 6.02 together, BAT could detect 20 of the 23 children with latex allergy. The combined use of ImmunoCAP with all the recombinant

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NRL allergens and BAT with rHev b 5, rHev b 6.01 and nHev b 6.02, enabled the identification of NRL allergy in 22 of 23 patients. There is a positive and significant correlation between sensitization to Hev b 5 and the number of interventions. BAT and allergen-specific IgE determination could be used as first-line *in vitro* diagnostic tests in patients with NRL allergy.

The prevalence of natural rubber latex (NRL) allergy in the general population is only around 1%, but it is still an important problem in several risk groups. The most important risk groups are workers in the healthcare area and those who need to use latex gloves as a mean of protection (cleaners, greenhouse workers, etc.), children with spina bifida or other congenital malformations who require surgical interventions and atopic individuals in general. Patients with latex allergy often have other concomitant allergies to fruit and different foodstuffs, many of which are caused by cross-reactivity.

Over the last few years, there have been considerable advances in the identification and characterization of some important NRL allergens (1, 2). Healthcare workers (HCW) with latex allergy have been shown to produce immunoglobulin E (IgE) antibodies mainly against the 16 kDa protein Hev b 5, and against the precursor protein prohevein (Hev b 6.01; 20 kDa) and its N-terminal region hevein (Hev b 6.02; 4.7 kDa) (3–5). On the contrary, children with spina bifida or other congenital anomalies who require frequent medical explorations early in their life and children with a history of multiple surgical interventions have IgE antibodies against the 14.6 kDa protein Hev b 1 (6–8), and Hev b 3, of 23/27 kDa (6). Both particle-bound allergens are considered to be major allergens in this group of patients and, together with Hev b 5 and Hev b 6.01, form the most important group of NRL allergens. Hence, Hev b 5 is recognized by 92% of patients allergic to NRL in general and by 56% of spina bifida patients allergic to NRL (9).

In addition, Hev b 13 (early nodule-specific protein), a 42–46 kDa protein, appears to be an important allergen in spina bifida patients and HCWs, in whom prevalences of 75% and 87%, respectively, have been reported (10, 11).

Paediatric patients with NRL allergy who have not undergone surgery are considered to present similar sensitization to the same allergens as the adult population (12).

There are no documented studies that describe NRL sensitization in children with a history of surgical intervention but without any congenital malformation (urogenital anomalies, spina

bifida, etc.), although some authors have studied NRL allergy in children without a history of surgical intervention (13–15).

When studying the sensitization route, children with spina bifida and other children requiring multiple surgical interventions are exposed to NRL or other materials used during and after the surgical intervention via direct contact with the mucosa. However, children without interventions are exposed to non-medical NRL products such as balls, pacifiers, latex toys and elastic bands (15), and the sensitization route is either cutaneous or inhalant. This difference in the route of sensitization could be associated with the different sensitization patterns mentioned above.

The diagnosis of NRL allergy is based on the complete clinical history which associates the presence of clinical symptoms with exposure to NRL, but this presumed diagnosis should be confirmed by skin prick tests and/or *in vitro* techniques (16). Although skin prick tests are considered to be the most useful diagnostic tool, their accuracy and reliability can be influenced by a lack of appropriate standardization (17). Moreover, these involve a degree of risk for the patient, especially those who have had a previous anaphylactic reaction.

In one of our previous studies, we confirmed the value of the Basophil Activation Test (BAT) for the *in vitro* diagnosis of NRL allergy (18).

The aim of this work was to evaluate the sensitization profile to single NRL allergens in children without spina bifida and without repeated surgical interventions, by using different single recombinant and natural latex allergens in two analytical techniques: specific serum IgE quantification and flow cytometry determination of activated basophils expressing CD63, after stimulating cells from patients with NRL allergens.

Patients and methods

A total of 23 patients (12 boys and 11 girls) aged between 5 and 18 yr (mean age: 11.65 yr) and 10 healthy children (seven boys and three girls), also between 5 and 18 yr, with a mean age of 12.8 yr were selected.

Table 1. Data of patients with NRL allergy

Patient	Age (yr) /sex	Atopy	Pollinosis	Symptoms	Latex-fruit syndrome*	SI	Conjunctival provocation (concentration + test; $\mu\text{g/ml}$)	In-use test (+test time; min)
1	6/M	Yes	No	U/AE	No	0	1.6	10
2	7/F	Yes	No	U/AE	No	0	Not done	10
3	8/F	No	No	U/AE	No	2	40	30
4	14/F	No	No	U/AE – RC/BA	Yes	3	1.6	20
5	12/M	No	No	Anaphylaxis	Yes	2	1.6	10
6	14/F	Yes	Yes	U/AE	Yes	3	Not done	Not done
7	15/F	No	No	Anaphylaxis	Yes	1	8	10
8	5/F	No	No	U/AE	No	3	Not done	20
9	9/M	No	No	U/AE – RC/BA	No	2	Negative	30
10	14/M	No	No	RC/BA	No	1	8	10
11	15/M	No	No	U/AE	No	2	8	30
12	15/M	Yes	Yes	RC/BA	No	1	Negative	30
13	11/M	Yes	Yes	U/AE – RC/BA	No	4	40	10
14	17/M	Yes	No	U/AE – RC/BA	No	1	40	20
15	15/F	Yes	No	Anaphylaxis	Yes	0	40	30
16	16/M	No	No	RC/BA	Yes	2	8	30
17	13/M	No	No	U/AE – RC/BA	Yes	1	1.6	10
18	10/F	No	No	U/AE – RC/BA	No	5	Negative	10
19	7/F	Yes	No	U/AE	Yes	0	8	10
20	7/M	No	No	U/AE – RC/BA	No	4	Negative	30
21	17/F	Yes	Yes	U/AE	No	0	Negative	20
22	6/M	No	No	U/AE – RC/BA	No	6	8	10
23	15/F	No	Yes	RC/BA	No	7	40	20

M, male; F, female; RC, rhinoconjunctivitis; BA, bronchial asthma; U, urticaria; AE, angio-oedema; SI, number of surgical interventions; NRL, natural rubber latex; IgE, immunoglobulin E.

*Sensitization to fruits (chestnut, kiwi, avocado) determined by clinical symptomatology (positive skin prick test or positive specific IgE).

The following inclusion criteria were used for patients: clinical history of NRL-induced urticaria and/or angio-oedema, rhinoconjunctivitis, bronchial asthma or anaphylaxis, with positive skin prick tests and NRL-specific IgE.

All the controls had negative skin prick tests against latex and tolerated latex contact. Detailed clinical data for all patients are shown in Table 1.

After obtaining informed consent from the parents, skin prick tests were performed with a commercial extract of ammoniated latex with latex protein concentration of 500 $\mu\text{g/ml}$ (ALK-Abello, Madrid, Spain), positive control (glycerinated histamine 10 mg/ml) and negative control (glycerosaline solution) as well as with a battery of aeroallergens and common food allergens in our setting, including food products having cross-reactivity to NRL (kiwi, chestnut, banana, fig, potato and tomato). Patients with a wheal > 3 mm and erythema, compared with the negative control, were considered as positive.

Atopy was defined as at least one positive skin prick test with the common allergens tested (19).

Recombinant and natural allergens

Recombinant maltose-binding protein (MBP)–rHev b fusion proteins [rHev b 1 (20), rHev b 5

(21), rHev b 6.01 (22), rHev b 8 (23) and rHev b 11 (24)] were expressed in *Escherichia coli* and purified by affinity chromatography as described. In the case of rHev b 3 and rHev b 9 the expression and purification was performed as described by Rihs et al. (20). Natural latex hevein, Hev b 6.02, was purified as described in the study by Chen et al. (5).

In vivo test

Conjunctival and in-use NRL provocation tests were carried out.

Conjunctival provocation tests were performed by the same investigator using a method based on the one described by Möller et al. (25) and Dreborg (26). Once we had ensured that the patient had not received any medication that could alter the test results (27), the test was started using a saline solution as the negative control. Concentrations ranging from 0.32 to 40 $\mu\text{g/ml}$ of a crude NRL extract were used (ALK-Abello).

The latex glove provocation test (in-use test) was carried out by placing a latex glove on the patient's previously moistened hand and inspecting the hand for the appearance of pruritus, erythema and local welts 10, 20 and 30 min after the start of the test, recording the time when these appeared. The test ended when local or

systemic manifestations were observed, or 30 min after the start of the test.

In vitro test

Specific IgE antigen. Specific IgE serum allergen determination was carried out in all patients and controls with the recombinant NRL allergens: rHev b 1, rHev b 2, rHev b 3, rHev b 5, rHev b 6.01, rHev b 6.02, rHev b 8, rHev b 9 and rHev b 11 according to the method described earlier (28, 29). Recombinant NRL allergens were coupled to individual ImmunoCAPs. The MBP was also coupled on ImmunoCAPs and served as a negative control. The individual NRL allergen profile was determined with sera and the ImmunoCAPs using the Pharmacia UniCAP-System (Pharmacia Diagnostics AB, Uppsala, Sweden) (data > 0.35 kU/l were considered as positive).

Basophil Activation Test. The BAT was performed with whole latex extract and with the recombinant antigens rHev b 5 and rHev b 6.01, as well as with the natural antigen Hev b 6.02, and MBP as control, at two concentrations (0.5 and 0.05 µg/ml). These were chosen as the optimal concentrations after a dose-response study was carried out in 10 HCWs diagnosed with NRL allergy, with positive skin prick test and specific IgE against latex, in whom final concentrations of rHev b 5, rHev b 6.01, MBP and nHev b 6.02 of 5, 0.5 and 0.05 µg/ml were tested.

In the BAT, proteins with the widest range of positivities in the specific IgE determination were used. rHev b 1 was not assayed in the BAT, because only three of the 23 patients presented specific IgE antibodies to this protein. For this same reason, rHev b 2, rHev b 3, rHev b 8, rHev b 9 and rHev b 11 were not used in the BAT, as they presented a positivity below 18%.

To evaluate the BAT results against recombinant fusion proteins, MBP-dependent activation results were subtracted from the activation results induced by the MBP-rHev b fusion proteins. BAT was considered as positive when at least one of the concentrations tested was positive.

Positivity criteria for BAT were those used in a previous study: a percentage of basophils activated against the major antigen or equal to 10%, and a stimulation index higher than 2 (18).

Statistical study

Data were analysed using the spss 11.0 statistical programme. As the variables had a normal distribution, the results were expressed as mean values and standard deviations.

Qualitative data were compared using the chi-squared test, using Yate's correction where necessary. In the case of samples with values below 20 or with an expected number of cases under 5, Fisher's exact test was used.

Concordance between the diagnostic tests was determined using the Kappa index. For all statistical data a significance level of p-values below 0.05 was established.

Results

Specific IgE against NRL and recombinant NRL allergens

Specific IgE was positive against NRL for all children with latex allergy and negative against the carrier protein MBP in all cases studied.

For the different recombinant allergens studied, the results are shown in Table 2. Noteworthy results correspond to a positivity for rHev b 5 in 10 children, for rHev b 6.01 in 16 and for rHev b 6.02 in 15 children sera. Specific IgE to rHev b 8 was found in four sera of the children. No specific IgE responses were found when using rHev b 2-ImmunoCAP.

We only found significant differences in sensitization against rHev b 5 in children with two or more surgical interventions compared with the non-intervened group or those with only one intervention ($p < 0.05$), and no statistically significant differences were detected for the other allergens tested.

Specific IgE was positive against at least one of the nine recombinant allergens tested in 21 of 23 cases. In 18 children, we found specific IgE against rHev b 5 and rHev b 6.01 and/or rHev b 6.02. Of the five negative sera for these recombinant allergens (patient 8, 12, 19, 20 and 21; Table 2), three were positive for other recombinant allergens: in one case for rHev b 1 and rHev b 3 (patient 8), in two cases for rHev b 8 and rHev b 9 (cases 12 and 21, one of which was also positive for rHev b 11). The other two patients (cases 19 and 20) were negative for all the recombinant allergens tested using this technique.

None of the 10 control children sera showed specific IgE to NRL and to the recombinant allergens. Finally, the sensitivity as well as the specificity of specific IgE for NRL (k82) were 100%.

Basophil Activation Test against NRL recombinant and natural allergens

The BAT against whole NRL extract was positive in 22 of 23 children; against rHev b 5 in 14 of the patients studied; against rHev b 6.01 in

Table 2. Allergen-specific IgE values of patients with NRL allergy

Patient	Latex (kU/l)	rHev b 1 (kU/l)	rHev b 2 (kU/l)	rHev b 3 (kU/l)	rHev b 5 (kU/l)	rHev b 6.01 (kU/l)	rHev b 6.02 (kU/l)	rHev b 8 (kU/l)	rHev b 9 (kU/l)	rHev b 11 (kU/l)
1	32.50	—	—	—	13.80	8.90	7.01	—	—	—
2	77.30	—	—	—	—	77.40	68.8	—	—	—
3	21.70	—	—	—	25.00	3.06	0.85	—	—	—
4	1.86	0.68	—	—	—	—	2.63	—	—	—
5	16.20	—	—	—	10.10	1.54	5.37	—	—	—
6	29.60	0.35	—	—	—	21.70	16.6	19.5	—	—
7	98.90	—	—	—	71.20	41.80	28	—	—	—
8	93.60	17.10	—	65.8	—	—	—	—	—	—
9	9.57	—	—	—	7.99	1.24	0.64	—	—	—
10	2.52	—	—	—	—	0.79	3.16	—	—	—
11	3.16	—	—	6.65	—	4.48	—	—	—	—
12	37.30	—	—	—	—	—	—	65.3	23.7	—
13	36.00	—	—	—	21.9	1.03	—	46.50	—	—
14	1.98	—	—	—	—	1.92	1.92	—	—	0.48
15	13.00	—	—	—	—	11.80	11.9	—	—	—
16	5.48	—	—	—	3.11	2.77	2.95	—	—	—
17	24.70	—	—	—	17.4	6.06	5.63	—	—	—
18	4.61	—	—	4.55	0.43	—	—	—	—	—
19	0.50	—	—	—	—	—	—	—	—	—
20	0.54	—	—	—	—	—	—	—	—	—
21	8.88	—	—	—	—	—	—	11.60	11.60	11.60
22	21.50	—	—	—	15.7	1.66	3.59	—	—	—
23	1.66	—	—	—	—	0.79	0.73	—	—	—
No positive cases	23	3	0	3	10	16	15	4	2	2
Positive cases (%)	100	13.04	0	13.04	43.48	69.56	65.22	17.39	8.69	8.69

—, negative result; IgE, immunoglobulin E; NRL, natural rubber latex.

seven cases and against nHev b 6.02 in 19 children (Table 3). In all the control subjects, the results using BAT were negative for each of the allergens tested.

BAT sensitivity to NRL was 95.65% and the specificity for each of these was 100%.

If we use allergens rHev b 5, rHev b 6.01 and nHev b 6.02 together, BAT could detect 20 of the 23 children with latex allergy. Natural Hev b 6.02 detects a greater number of cases in BAT than the rHev b 6.01 (19 vs. 7). On the contrary, in three cases we obtained a negative response to anti-IgE but a positive allergen-specific response.

BAT vs. ImmunoCAP method

The combined use of ImmunoCAP with all the recombinant NRL allergens and BAT with rHev b 5, rHev b 6.01 and nHev b 6.02, enabled the identification of NRL allergy in 22 of 23 patients. rHev b 5 was positive with one of the two techniques in 15 of 23 children, rHev b 6.01 in 17 children and Hev b 6.02 in 21. Using only these three allergens with the two techniques, we could detect 21 of the 23 children with NRL allergy.

The correlation between the two techniques was positive and significant for the two concentrations of rHev b 5 in BAT ($r = 0.55$, $p = 0.007$; $r = 0.64$, $p = 0.001$), and there was no signifi-

cant correlation between the two techniques for the other allergens tested (Table 4). On the contrary, there was a good concordance between BAT and ImmunoCAP, of 73.9% for rHev b 5, 52.2% for rHev b 6.01 and 65.2% for nHev b 6.02. For NRL, concordance between the two techniques was 95.6%.

There is also a positive and significant correlation between sensitization to Hev b 5 and the number of interventions ($r = 0.571$, $p = 0.004$).

With the combined use of IgE determination with all the recombinant allergens and BAT with rHev b 5, rHev b 6.01 and nHev b 6.02, we established the NRL allergen profile of 22 of 23 patients (Fig. 1). The five patients sensitized to Hev b 1 and/or Hev b 3 had undergone more than three surgical interventions.

In sera of all children with latex-fruit syndrome-specific IgE to nHev b 6.02, but not to rHev b 11 were detected. The patients sensitized to Hev b 8, Hev b 9 and/or Hev b 11 were atopic. The four patients presenting a positive response to the NRL profilin (Hev b 8) were allergic to pollen.

Discussion

Many allergenic latex proteins have been isolated and purified and recombinant allergens were

Table 3. BAT values vs. latex and different recombinant allergens (results expressed in percentage basophil activation)

Patient	BAT (basal)	BAT (A-IgE)	BAT vs. latex (0.125 mg/ml)	BAT vs. latex (0.031 mg/ml)	BAT vs. rHev b 5 (0.5 µg/ml)	BAT vs. rHev b 5 (0.05 µg/ml)	BAT vs. rHev b 6.01 (0.5 µg/ml)	BAT vs. rHev b 6.01 (0.05 µg/ml)	BAT vs. nHev b 6.02 (0.5 µg/ml)	BAT vs. nHev b 6.02 (0.05 µg/ml)
1	1	62	60	55	—	23	18	—	—	70
2	5	58	29	39	—	—	23	17	53	57
3	0	89	—	43	23	—	—	—	—	—
4	17	68	68	61	49	67	51	67	68	74
5	1	48	77	83	86	64	—	—	89	93
6	1	29	68	63	—	—	—	—	—	33
7	3	23	57	66	51	46	36	13	52	63
8	7	31	49	64	—	45	—	—	61	70
9	0	9	29	61	—	—	—	—	24	23
10	1	24	79	80	21	—	10	48	67	78
11	3	2	37	45	39	54	—	14	51	46
12	2	62	69	62	—	—	—	—	—	—
13	3	81	35	45	38	67	—	—	19	17
14	4	48	—	16	—	—	—	—	—	—
15	0	64	47	41	—	—	—	15	42	52
16	0	4	13	14	11	12	—	—	13	11
17	15	48	85	85	67	65	—	—	84	84
18	1	35	60	61	65	55	—	—	64	66
19	1	63	18	11	—	—	—	—	—	10
20	4	76	—	—	—	—	—	—	—	—
21	19	67	89	88	—	—	—	—	41	37
22	2	24	20	25	42	46	—	—	30	32
23	8	19	32	36	10	—	—	—	—	37
No positive cases		22	14	7	19					
Positive cases (%)		95.65	60.87	30.43	82.61					

—, negative result; BAT, Basophil Activation Test; IgE, immunoglobulin E.

Table 4. Concordance and correlation between BAT and allergen-specific IgE to latex and rHev b 5, rHev b 6.01 and n/rHev b 6.02

<i>In vitro</i> technique – allergen	Concordance (%)	Correlation, r (p-value)	
		First concentration	Second concentration
BAT – rHev b 5; CAP – rHev b 5	73.9	0.550* (0.007)	0.644* (0.001)
BAT – rHev b 6.01; CAP – rHev b 6.01	52.2	0.363 (0.088)	0.243 (0.265)
BAT – nHev b 6.02; CAP – rHev b 6.02	65.2	0.172 (0.433)	0.232 (0.287)
BAT – latex; CAP – latex	95.6	0.205 (0.348)	0.253 (0.245)

*Correlation is significant at 0.01 level (bilateral; Pearson's correlation).
BAT, Basophil Activation Test; IgE, immunoglobulin E.

produced. These allergens offer numerous advantages. The first investigations of reactivity of IgE antibodies against recombinant allergens have shown that most of these molecules have similar IgE epitopes than natural allergens and can be used for allergologic diagnosis and to determine the patient's specific IgE reactivity profile on a molecular level (28, 30, 31). One exception is the IgE-binding capacity of rHev b 2, which did not work in the recombinant form produced in *E. coli* (29), because of carbohydrate structure of Hev b 2. The diagnostic tests based on recombinant allergens could be used as one criterion to select patients for specific immunotherapy. Moreover, new types of vaccines based on recombinant allergens are also emerging.

The availability of recombinant allergens has enabled their application in previously existing diagnostic tests, such as specific IgE determination (7, 32) and BAT (33, 34).

The value of BAT has been demonstrated for the *in vitro* diagnosis of different allergic conditions (35–37), including latex allergy (18, 38), using recombinant allergens (30).

In this study, we have used for the first time two *in vitro* diagnostic techniques, BAT and specific IgE determination against recombinant and natural latex allergens, in the same study group of children with NRL allergy with or without surgical interventions. With flow cytometry to study basophil activation and antigen-specific IgE determination, we attempted to

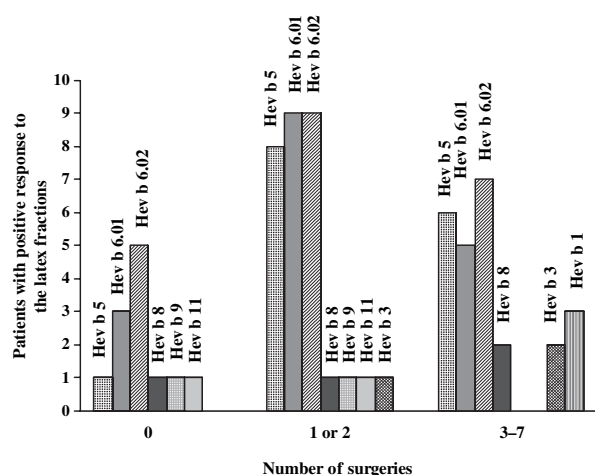


Fig. 1. Positive patients to the different latex fractions in relation to the number of surgeries.

identify the sensitization profile of this group of patients.

Based on our results, we can confirm that overall, these children are more frequently sensitized to Hev b 5, Hev b 6.01 and Hev b 6.02, according to specific IgE results and BAT. With the combined use of these two techniques with the allergens rHev b 5, rHev b 6.01 and nHev b 6.02, could be detected in 22 of the 23 cases. Patient 20, negative case with both techniques, had only one positive latex provocation test after 30 min and a low positive specific IgE response against NRL (0.54 kU/l). The specificity estimated for recombinant allergens in both techniques was 100%.

The behavioural differences between natural and recombinant Hev b 6.02 in the BAT could be due to a folding defect occurring, or due to the fact that the latter is a fusion protein and relevant epitopes could be shielded.

Similar to other studies (6–8), we also found sensitization only to Hev b 1 and Hev b 3 in multioperated patients. An interesting result in our study is the presence of a positive and significant correlation between sensitization to Hev b 5 and the number of surgical interventions. Our series, which includes children with and without surgical interventions, permitted us to demonstrate this finding, which was most significant in the different response of the two groups to rHev b 5, determined by both BAT and ImmunoCAP methods.

Other studies included non-multioperated (7, 17) or multioperated children in their series (6–8). In these studies, sensitization to Hev b 6.01 and Hev b 6.02 is common, regardless of the children's surgical history. In our study, all the

children with latex-associated fruit allergy are sensitized to hevein (Hev b 6.02). This finding agrees with the well-known phenomenon of cross-reactivity between hevein and some groups 3 and 4 plant defence proteins (PR 3 and 4) that present hevein domains, including class I chitinases, considered as panallergens responsible for latex-fruit syndrome (39).

The low sensitization obtained against the class I chitinase Hev b 11 is noteworthy (two of 23 patients). As reported by O'Riordain et al. (40) and Rihs et al. (24), Hev b 11 is another allergen from *Hevea* latex with a chitin-binding domain, with an IgE-binding capacity different from that of hevein Hev b 6.02.

All patients with NRL allergy and sensitization to Hev b 8 have a background of pollinosis. The most probable immunological explanation for this clinical association is a cross-reactivity due to profilins.

In relation to the values of these determinations, we would like to recommend to use rHev b 5 and nHev b 6.02 in BAT, because positive cases for rHev b 6.01 were also positive for nHev b 6.02 (Table 3).

In our study, three of the 23 patients with latex allergy did not respond by stimulation with anti-IgE (positive control) in BAT, but were not eliminated from the study given their positive allergen-specific response. Some authors confirmed a low response to anti-IgE, although they do not rule out the functional sensitivity to a given allergen (41).

The concordance between the two techniques was higher than 65% for Hev b 6.02 and 74% for Hev b 5. Purohit et al. (34) found that the concentrations of allergen-specific IgE antibody sensitivities are not necessarily associated in a study with recombinants of birch pollen allergen Bet v 1. On the contrary, we found a positive and significant correlation between the degree of basophil activation and specific IgE only for the rHev b 5 allergen. This allergen contains repeated IgE-binding domains, probably because this protein is one of the allergens mostly recognized by all patients with latex allergy, regardless of the risk group to which they belong (9).

This study is the first report to use and combine recombinant allergens against NRL in BAT and specific IgE test in children with NRL allergy with a history of multiple surgical interventions, demonstrating the diagnostic value of these two techniques.

Further, BAT and allergen-specific IgE determination could be used as first-line *in vitro* diagnostic tests in patients with NRL allergy.

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